

THE CONTROL OF REPRODUCTION IN DIPLOPTERA PUNCTATA (BLATTARIA)¹

FRANZ ENGELMANN

Department of Anatomy, Albert Einstein College of Medicine, New York 61, N. Y.

In cockroaches, as in many insect species studied, a hormone released by the corpora allata is necessary for the maturation of eggs (Scharrer, 1946; Engelmann, 1957). A variety of intrinsic and extrinsic factors, such as pregnancy, egg resorption, or involution of the corpus luteum (Lüscher and Engelmann, 1955; Engelmann, 1957), food supply (Scharrer, 1946; Johansson, 1955, 1958; von Harnack, 1958), and probably humidity and temperature are involved in the control of the function of the corpora allata. Furthermore, in some cockroach species, mating accelerates the growth rate of the eggs and, therefore, increases the total number of eggs produced during the lifetime of the animal (Griffiths and Tauber, 1942; Roth and Willis, 1956; Wharton and Wharton, 1957).

In *Diploptera punctata*, a viviparous cockroach, the influence of mating, an extrinsic factor, on egg maturation plays a more dominant role than in related species. In virgins, the maturation of the first batch of eggs takes several weeks or even months (Roth, personal communication), whereas after mating only 10 days are required for egg maturation (Roth and Willis, 1955). Since a variety of factors known to condition the activity of insect ovaries reach the gonads via brain and corpora allata (Scharrer, 1958) it seems probable that the stimuli exerted by the act of mating influence the ovary by the same route.

The present paper deals with the elucidation of the control mechanism governing female reproduction in *Diploptera*. Particular emphasis was given to the study of the pathways by which the mating stimulus reaches the ovary.

MATERIAL AND METHODS

All experimental animals were taken from a stock colony and maintained in finger bowls at room temperature of 22–26° C. They were fed dog chow and water. Daily records were kept of the reproductive activity of the females. The presence of a spermatophore in the bursa copulatrix of the female indicated mating, that of an egg case in the brood sac ovulation.

Experiments such as castration, allatectomy, excision of the gonapophyses, and severance of nerves were performed under CO₂ anesthesia. The nervi corporis cardiaci or the nervi corporis allati were severed either by frontal or occipital approach.

For the histological investigation of the corpora allata and for the determination of the completeness of the severance of nerves an organ complex consisting of brain, corpora cardiaca, and corpora allata was fixed either in Bouin-Duboscq's or in Zenker's fluid. The sections were stained in Gomori's chrome hematoxylin phloxin

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or in aldehyde fuchsin as modified by Halmi. The volume of corpus allatum tissue per one million nuclei was determined by a formula given in an earlier paper (Engelmann, 1957). The data obtained, expressed as mean values for both corpora allata of a given specimen, indicate the degree of activity of the corpora allata; these values were correlated with the observed length of the oocytes.

RESULTS

1. The cyclic activity of ovary and corpus allatum

In ovoviparous or viviparous cockroaches there is no space for additional eggs within the brood sac of the female during the development of the embryos. Egg maturation in the ovaries must, therefore, be temporarily suppressed. Since in other species of cockroaches egg maturation depends on the activity of the corpora allata, we first looked for a possible relationship between the structure of the corpora allata and ovarian activity in *Diploptera* during an interval encompassing the first pre-oviposition, first pregnancy, and second pre-oviposition periods. The results which are reported in the following paragraphs provided a basis for experimental work.

In *Diploptera* mating takes place a few minutes after emergence (Roth and Willis, 1955). Within 9.43 ± 0.22 days thereafter (53 animals observed in our laboratory) the largest oocyte of each ovariole grows from about 0.55 mm. to a size of approximately 1.5 mm. and then ovulates. Large amounts of yolk are deposited in the oocytes during their period of growth.

No eggs ripen in the ovaries during pregnancy, which follows ovulation and which lasts for 75.49 ± 1.47 days (45 animals; see also Willis *et al.*, 1958). There is merely a slight growth of the oocytes, but no yolk whatsoever is deposited until the second or third day before parturition. Then a more rapid growth of the oocytes accompanied by yolk deposition begins, so that on the day of parturition the largest eggs in the ovarioles measure about 0.87 mm. (Fig. 1). Within the next three or four days the eggs grow to full size; 5.98 ± 0.17 days after parturition these mature eggs ovulate (45 animals).

The activity of the corpora allata, as judged by the amount of cytoplasm, correlates with the activity of the ovaries. The relative and absolute amount of cytoplasm in these glands gradually increases during the first pre-oviposition period until a maximum of activity is reached at the fourth day after mating (Fig. 1). Values of less than 0.95 mm.³ of total tissue per one million nuclei indicate that this gland is inactive; more than 1.45 mm.³ tissue per one million nuclei represents a highly active gland. Between these figures there are varying degrees of activity. The cells of the corpora allata maintain a high activity for about four days, during which time most of the yolk is deposited in the oocytes. Shortly before ovulation, a decrease in the activity of the corpora allata is observed, so that on the day of ovulation the corpora allata contain only a small amount of cytoplasm.

During pregnancy, when the ovaries are quiescent, the corpora allata are inactive, as shown by histological criteria (Fig. 1; see also Table II, a); the distribution of the nuclei in the corpora allata is similar to that on the day of emergence, i.e., 0.84 mm.³ gland tissue per one million nuclei. Shortly before parturition an activation of the corpora allata can be observed which gradually increases until

the second or third day after parturition (Fig. 1). After reaching a peak of activity, the cytoplasmic content of the glands decreases. The corpora allata are nearly inactive again at the time of the second ovulation.

The second pre-oviposition period is three days shorter than the first. The growth rate of the oocytes, however, is about the same in both periods (Fig. 1). The shortening of the second period is merely due to the fact that yolk deposition in the second batch of oocytes begins already during the late days of pregnancy. The average peak of corpus allatum activity in the second reproductive period is below that of the first, but this difference is statistically insignificant. The reason for the variability of the data is, at least in part, that the onset of the second period of activity is not as clearly defined as that of the first period. Therefore, the

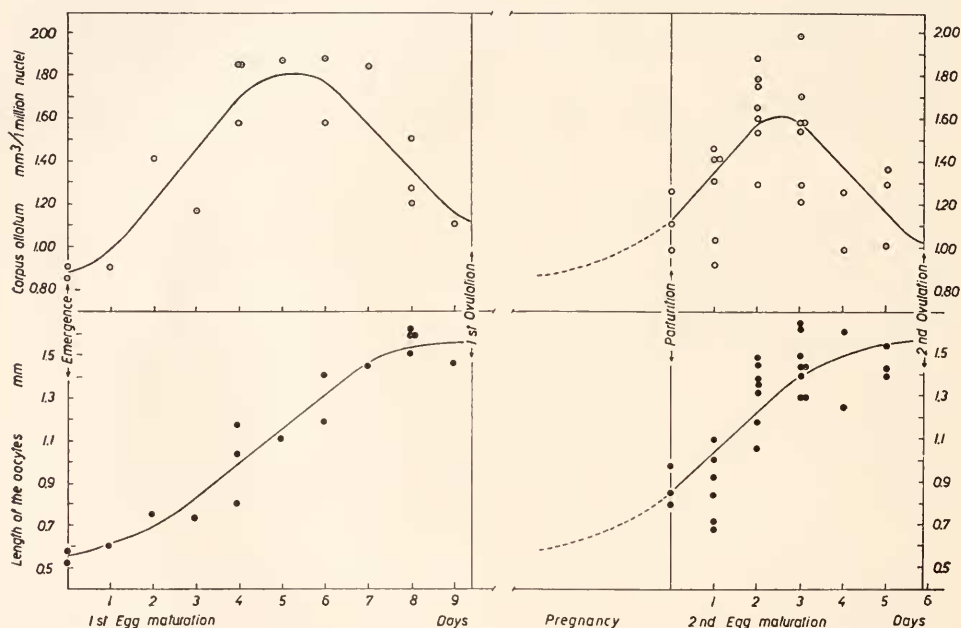


FIGURE 1. Diagram indicating the morphological changes signalling cyclic activity in the corpora allata of *Diploptera* (upper curves), which are correlated with the growth of the oocytes (lower curves) during the first and second pre-oviposition periods.

corpora allata of some females may have surpassed their highest activity when those of other females, fixed after the same interval, are still maximally active.

2. The function of the corpora allata in the adult female

The role of the corpora allata in the ovarian control of *Diploptera* was further elucidated by the removal of these glands. Allatectomy was performed two to four days after mating. In all nine animals thus operated the deposition of yolk in the oocytes beyond the stage characteristic of the day of operation was prevented. Twenty days after mating degeneration of most of the oocytes of the al-

lactectomized animals had begun. It appears that by the fourth day after mating not enough corpus allatum hormone has been released into the circulation to permit the completion of egg maturation. Thus in *Diploptera*, in the same way as in other species of cockroaches, egg maturation depends on the activity of the corpora allata not only during the initial phase, but also during the time when most of the yolk is being deposited in the oocytes.

The activity of the accessory glands of the female genital apparatus also depends on the function of the corpora allata, since no secretory material is detectable in the lumen of these glands in animals allatectomized shortly after mating. There is yet another type of secretory cell that seems to be under the control of the corpora allata as the following observation indicates. Around the sixth day after mating the spermatophore is extruded from the bursa copulatrix (53 animals). At the same time an aqueous fluid appears in the genital apparatus, which probably facilitates the extrusion of the spermatophore. In allatectomized females the spermatophore stays within the bursa, and the genital apparatus remains dry. In four normally mated females, in which for undetermined reasons the corpora allata did not become active, as shown by the failure of eggs to mature, the spermatophores remained in the bursa copulatrix for 18 days at which time the females were fixed for further investigation.

Thus it appears that the hormone released by the corpora allata serves at least three different functions in the adult female of *Diploptera*. It causes the deposition of yolk in the oocytes, it activates the accessory glands of the female genital apparatus, and it stimulates an as yet unknown type of cell presumably present in the bursa copulatrix, whose secretion facilitates the extrusion of the spermatophore. The function of the accessory glands and of the secretory cells in the bursa seems to be regulated directly by the corpora allata, since this mechanism is not disturbed by ovariectomy.

3. The control of the activity of the corpora allata by the brain

In *Diploptera*, as in many other insect species studied, the brain innervates the corpora allata via the nervi corporis allati, which are a component of the nervi corporis cardiaci. Severance of these nerves was attempted in 44 virgins of *Diploptera*, one to 10 days after emergence. Activation of the ovaries, *i.e.*, deposition of yolk in the oocytes, resulted in 39 of these animals, whereas in the five remaining roaches no ovarian activity was found. A histological study of the brain-corpora cardiaca-corpora allata complex of all 44 experimental animals was undertaken to check for the completeness of the severance of the nerves. In 16 of these animals, fixed nine to 33 days after operation, no severance or other disturbance of the innervation of the corpora allata could be detected histologically. This group of 16 virgins includes the five cases mentioned above that matured no eggs; the remaining 11 had matured their eggs. In all of these 16 virgins the corpora allata were inactive at the day of fixation. Obviously, the nerve severance had failed in this group. But how can egg maturation in 11 of these virgins be accounted for? Perhaps in these cases the nerves were squeezed or stretched during the operation, a situation which interfered with nerve conduction and thus led to temporary activation of the corpora allata. Apparently the brain must have resumed the inhibition of the corpora allata a few days later.

In 21 animals of the operated group an interruption of the nerves which innervate the corpora allata could be detected histologically; all of these animals had active corpora allata, as determined by histological criteria. In all, egg maturation took place as a result of the activation of the corpora allata within a period similar to that normally observed after mating. From this it seems that mating does not cause a higher activity of the corpora allata than that which results from severance of the nerves that transmit inhibitory impulses to the corpora allata. One might conclude, therefore, that mating prevents the flow of inhibitory nerve impulses. In the remaining seven animals, which also matured their eggs, the success of the operation could not be determined, because parts of the tissue were lost during the histological procedure. It appears that severance of the nervi corporis allati in virgins results in activation of the corpora allata, which indicates that in unmated young females the brain restrains the function of the corpora allata by way of the nervi corporis allati.

It was of further interest to determine whether the corpora allata severed from the restraining center in the brain remain active permanently or only for a period sufficient to induce the maturation of one batch of eggs. In order to test this point, 15 virgins with severed nervi corporis allati were fixed 14 to 31 days after their first ovulation had taken place. In eight of these animals only one egg maturation had occurred; the size of the next oocytes in line in the ovarioles was about 0.40 mm. at the time of fixation. This size is comparable to the measurements in pregnant females at a similar interval after ovulation. The corpora allata of these eight animals were inactive as determined by histological criteria. The innervation of the corpora allata in these animals seems to have been incomplete. A few intact nerve fibers were observed in the sections. It may be that these had been squeezed and thus prevented from inhibiting the corpora allata for a short time, or these nerve fibers may have regenerated and thus may have resumed inhibition of the corpora allata. This might have been the case also in four additional virgins in which egg maturation had begun a second time, but had not progressed beyond an initial phase; here the largest oocytes measured on the average 0.62 mm. 20 to 30 days after the preceding ovulation, whereas in normal pregnant females, after a similar interval, the oocytes measured only about 0.40 mm. In these operated virgins the resumption of the inhibition of the corpora allata may have taken more time and, therefore, a second growth period of the oocytes had been initiated. Only in three specimens, in which the separation of the corpora allata from the brain was complete at the time of fixation (interval over 40 days), the glands were active (average of 1.32 mm.³ gland tissue per million nuclei). In these three animals, the corpora allata had induced the growth of a second batch of eggs to full size (1.50 mm.) within 24 to 30 days after the first ovulation.

Thus, severance of the corpora allata from the restraining center in the brain results in a sustained activity of the glands. This activity persists at least long enough for two successive batches of eggs to mature. In the three animals with completely severed corpora allata the maturation of the second batch of eggs took considerably longer than that of the first. This may have two reasons. In the first place yolk deposition begins when the oocytes measure about 0.55 mm. which means that the oocytes next in line after ovulation have first to grow from about 0.35 mm. to 0.55 mm. This initial growth of the oocytes results in a prolongation

of the pre-oviposition period which immediately follows ovulation. A second factor may be a restraining influence coming from the corpus luteum which, during its involution, seems to inhibit the growth of the next oocyte in line, as was reported in *Leucophaea* (Engelmann, 1957).

4. The activation of the corpora allata by afferent stimuli

In mated females of *Diploptera* the growth of the oocytes accompanied by the deposition of yolk began about one day after emergence, and was completed within 10 days thereafter (53 animals; see also Table I, a). Among 45 virgins, on the other hand, the shortest interval in which egg maturation occurred was 37 days (one animal); at 60 days after emergence eight additional virgins had ovulated.

TABLE I
Experiments during first pre-oviposition period

	Operation (days after emergence)	Fixation		Number of animals	Corpus allatum (mm. ³ /1 million nuclei)	Ovary (length of the largest oocytes in mm.)
		(Days after operation)	(Days after emergence)			
a. Control: mated females	—	—	4-9	12	1.49±0.08	1.32±0.08
b. Severance of ventral nerve cord. No mating	1-3	3-23	4-24	8	0.95±0.03	0.60±0.02
c. Severance of ventral nerve cord followed by mating	1	6-20	7-21	8	0.85±0.02	0.61±0.02
d. Artificial mating stimuli	5-19	3-14	17-26	8	1.03±0.08	0.72±0.04
e. Excision of gonapophyses	1-5	8-11	9-14	12	1.33±0.14	1.11±0.10
f. Control: virgin females	—	—	5-31	8	0.84±0.02	0.59±0.01

Numbers following \pm are standard errors.

Of the remaining 36 animals 13 virgins ovulated within a period of two to five months, whereas 14 animals had not ovulated when they either died or were discontinued for other reasons. Nine virgins had not ovulated even after five months. It is not definitely known whether or not all virgins eventually produce mature eggs without mating. It is obvious, however, that mating is essential for a normal growth rate of the oocytes maturing after emergence.

One may now ask, whether mating is equally essential to induce the normal growth rate of eggs maturing after the first parturition. Therefore, 24 females were isolated at parturition. The next ovulation occurred 5.83 ± 0.28 days thereafter. In eight additional females, in which mating was allowed to take place on the first day after parturition, the pre-oviposition period took 5.75 ± 0.37 days. Thus, mating does not accelerate the growth of the eggs maturing after parturition. From this it appears that giving birth may serve as a stimulus replacing that of

mating from the induction of egg maturation at a normal rate. Furthermore, there is sufficient viable sperm stored in the spermathecae from the mating before the first pregnancy, so that additional batches of eggs can be fertilized.

If parturition activates the corpora allata and consequently the ovaries, how is it possible that yolk deposition in the second batch of oocytes begins a few days before parturition (Fig. 1)? The following observation may give an answer to this question. During the late days of pregnancy, when the abdomen of the female is distended by the ootheca, the animal rhythmically contracts the abdomen. The resulting movements of the egg case in the genital apparatus presumably initiate the activity of the corpora allata occurring before parturition. The observation that mating, as well as parturition, and presumably movements of the egg case in the genital apparatus, activate the corpora allata suggests that sensory receptors located in the genital apparatus receive those stimuli. It is likely that movements of the young hatching within the vagina (Roth and Willis, 1955) enhance the

TABLE II
Experiments with pregnant females

	Operation (days after ovulation)	Fixation		Number of animals	Corpus allatum (mm. ³ /1 million nuclei)	Ovary (length of the largest oocytes in mm.)
		(Days after removal of ootheca or parturition)	(Days after ovulation)			
a. Control: pregnancy	—	—	4-62	11	0.83±0.02	0.40±0.01
b. Removal of ootheca	0-41	3-31	19-58	13	0.95±0.03	0.56±0.06
c. Removal of ootheca followed by mating (within 4 to 6 days)	35-38	8-15	45-50	5	1.35±0.12	1.20±0.19
d. Control: parturition	—	4-5	—	5	1.10±0.08	1.14±0.06

Numbers following \pm are standard errors.

activity of the corpora allata resulting from the rhythmical contraction of the abdomen prior to parturition. It is not known, however, whether the initial activation of the corpora allata by the movement of the egg case in itself would be sufficient to bring about complete egg maturation. From the facts reported here and in the preceding paragraphs it appears that in *Diploptera* afferent stimuli are solely responsible for the induction of a normal growth rate of the oocytes in the ovaries.

This conclusion was further substantiated by an additional experiment. After the removal of the egg cases from five pregnant females maturation of the next batch of eggs took at least 41 days when no mating was permitted. On the other hand, if mating was allowed after egg case removal, the oocytes matured within 9 days after mating (Table II, c). The time required for the maturation of the eggs after removal of the egg case and subsequent mating, and that after mating following emergence are practically the same. Again, an external factor accounts for the induction of egg maturation at a normal rate.

If stimuli, received in the genital apparatus during mating and parturition, activate the corpora allata, a transmission of these stimuli to the brain and corpora allata via the ventral nerve cord has to be postulated. In order to test this point the ventral nerve cord was severed in 16 virgins 1–3 days after emergence. Eight of these animals were allowed to mate afterwards, whereas another eight animals were kept isolated as controls. None of the operated and mated females showed maturation of the eggs. The corpora allata must have remained inactive and, consequently, in these animals yolk deposition was completely prevented (Table I, c). The same was the case in the eight females, which did not mate after severance of the abdominal nerve cord (Table I, b). From these experiments one may conclude that stimuli received in the genital apparatus during the act of mating, and presumably also during parturition, are transmitted via the ventral nerve cord to the brain and corpora allata.

Further experimental proof for the conclusion that stimuli are received in the genital apparatus seemed desirable. Therefore, a small artificial spermatophore, made of glass, was pushed into the bursa copulatrix of virgins (Table I, d). Most of these artificial spermatophores remained in the bursa copulatrix for the duration of the experiment, but in some cases they were extruded and had to be replaced. A few days after successful implantation of the "spermatophores" the animals were sacrificed in order to check the degree of maturation of the oocytes and to determine histologically the state of the corpora allata. Among eight animals five had begun to deposit yolk in the oocytes. This result is not as clear-cut as that after mating (Table I, a), which might be due to the fact that a glass spermatophore is too smooth to afford sufficient stimulation. Furthermore, normal mating may last up to several hours during which time the movements of the male provide a more efficient stimulation of the genital apparatus than that caused by deposition of a smooth artificial glass spermatophore.

Apparently mating alone, without the deposition of a spermatophore, also induces activity of the corpora allata (Roth, personal communication). On the other hand, from the fact that yolk deposition in some of the experimental animals was induced by artificial mating, it appears that mechanical stimulation during the act of mating or during parturition tends to activate the corpora allata and in turn to induce maturation of the oocytes.

As stated above, it was thought that sensory receptors on the gonapophyses of *Diploptera* are involved in the perception of these mechanical stimuli. For this reason in 12 virgins all three pairs of gonapophyses were excised in order to prevent the perception of stimuli, when mating was allowed afterwards. However, none of the virgins thus operated upon accepted a male within the duration of the experiment. Nevertheless of the 12 operated females, 10 were found to have deposited yolk in the oocytes after a few days; in some females the eggs had reached nearly full size (Table I, e). Probably the afferent nerves were stimulated by the excision of the gonapophyses and this resulted in activation of the corpora allata and of the ovaries. The conclusion that sensory receptors on the gonapophyses receive the stimuli during the act of mating or parturition seems, therefore, justified. This experiment does not exclude the possibility that other parts of the genital apparatus supplied with sensory receptors may also be involved in the perception of mating stimuli. As the following experiment shows, this proved actually

to be the case. In three virgins with gonapophyses excised shortly after emergence mating eventually took place 21, 28, and 52 days after operation. Nine to ten days later, *i.e.*, after an interval normally required for egg maturation in mated females, ovulation took place. Thus, we conclude that sensory receptors on the gonapophyses and on other parts of the genital apparatus receive the stimuli exerted by mating and parturition.

5. The effect of mature eggs on the corpora allata

In other species of roaches mature eggs seem to exert an inhibitory effect on the corpora allata (Engelmann, 1957). The question arises whether in *Diploptera* mature eggs, either before ovulation or during embryonic development in the brood sac, likewise inhibit the corpora allata in their function. For this reason, the egg cases were removed from the brood sac during anesthesia in 13 females of *Diploptera*, at different stages of pregnancy. These animals were kept isolated to prevent mating and were fixed three to 31 days thereafter in order to check the degree of activity of the corpora allata and of the ovaries. None of these 13 females matured their eggs in the ovaries within the tested period (Table II, b). Additional five females, in which the oothecae were removed three to five days after ovulation, were kept until the next ovulation occurred. This took place 42, 51, 54, 57, and 71 days after the removal of the oothecae. Thus, the period required for egg maturation after removal of the egg case is roughly comparable to that observed in virgins. In other words, in this experimental series in which appropriate afferent stimulation was lacking, the corpora allata were retarded in their activity, but not completely suppressed.

By contrast, egg maturation is inhibited throughout the period of pregnancy which in *Diploptera* lasts 75 days. Therefore, the absence of mating or equivalent stimuli alone would not account for this complete state of quiescence in the ovary which in turn signals complete inactivity of the corpora allata. An additional inhibitory action seems to come from the ootheca and, in analogy with the known situation in *Leucophaea*, it may be due to a humoral factor.

As to the mechanisms which, in the absence of appropriate stimuli such as mating, keep the corpora allata of adult females of *Diploptera* partially suppressed, nothing can be added to the already discussed inhibitory nervous link between brain and corpora allata. It may well be that the factor eliciting this inhibition acts independently of the inhibitor released by the egg case.

Further support for the concept that a restraining factor from the eggs is not the sole or even primary afferent link governing the inhibition of the corpora allata of *Diploptera* was obtained by castration experiments. Eleven females were ovariectomized two to three days after mating. Yolk had already been deposited in the oocytes, which indicated that the corpora allata had been activated by mating. Twelve to 44 days after this operation the females were fixed in order to check histologically the activity of the corpora allata. The glands were completely inactive as indicated by the calculation of an average of 0.86 mm.³ of gland tissue per million of nuclei. From this it again appears that an as yet unknown mechanism residing outside the ovary inhibits the corpora allata. These findings in *Diploptera* are in contrast to observations in several other species of insects including *Leucophaea* (von Harnack and Scharrer, 1956) where ovariectomy results in hyperactivity of the corpora allata.

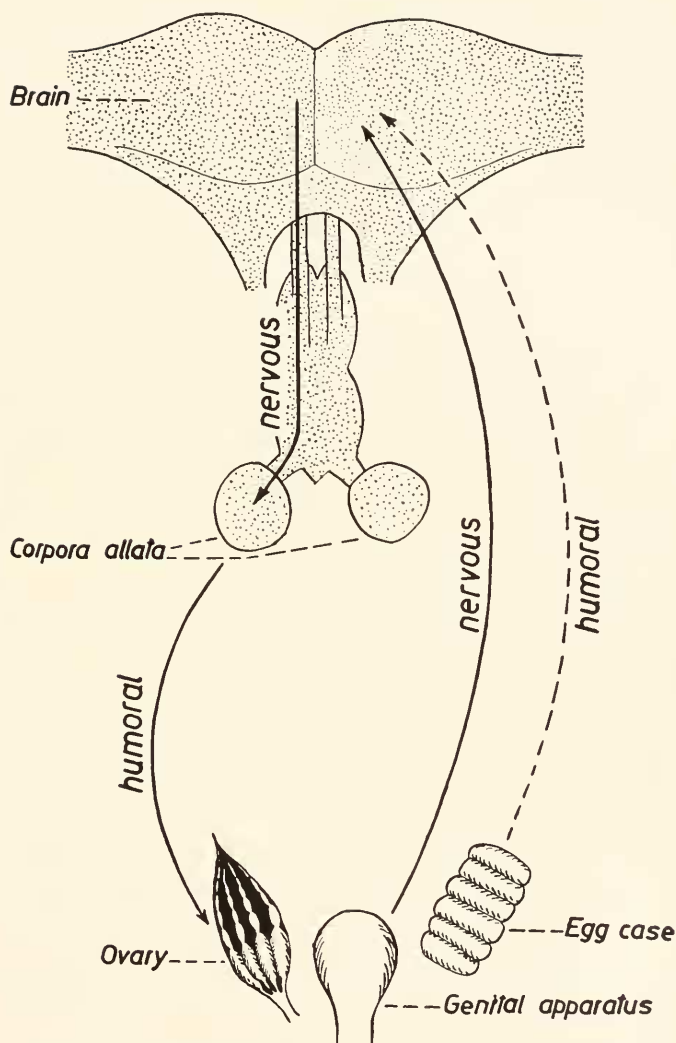


FIGURE 2. Diagram illustrating the control of reproduction in *Diploptera*. Stimuli exerted by mating or parturition, received in the genital apparatus, are transmitted to the brain where they inhibit the normal flow of restraining impulses from the brain to the corpora allata. The corpora allata then release gonadotropic hormone which induces egg maturation. During pregnancy, complete inhibition of the corpora allata is accomplished, presumably by a humoral principle originating in the developing eggs. For further details see text.

DISCUSSION

The control of egg maturation in the viviparous cockroach, *Diploptera*, depends on the following sequence of events (Fig. 2): Stimuli exerted by mating, which are received by sensory receptors on the gonapophyses or other parts of the genital apparatus, ascend via the ventral nerve cord to the brain and act to inhibit the normal flow of restraining impulses from the brain to the corpora allata. The corpora

allata then release a hormone that induces the maturation of the eggs. Parturition provides stimuli adequate for eliciting egg maturation in the second or subsequent cycles, and no mating is then required. In virgins, the corpora allata seem to be completely inhibited at first, but become slowly activated after some time and eventually induce egg growth. During pregnancy another controlling factor originating in the ootheca makes the inhibition of the corpora allata complete.

As in *Leucophaea* (Scharrer, 1952; Engelmann and Lüscher, 1956), and in *Oncopeltus* (Johansson, 1958), the brain in *Diploptera* restricts the function of the corpora allata by way of the nervi corporis allati. This can be concluded from the fact that severance of these nerves in the species mentioned results in persistent activity of the glands.

The fact that the brain inhibits the corpora allata in *Leucophaea* and *Diploptera* at certain periods suggests that extrinsic and intrinsic "information" is integrated in the brain and then passed on as messages to the corpora allata. In *Leucophaea* the presence of mature eggs in the brood sac causes the brain to inhibit the corpora allata by way of the nervi corporis allati during pregnancy. No eggs mature in the ovary during this period. On the other hand, in *Diploptera*, the brain converts stimuli received in the genital apparatus with the result that inhibitory impulses to the corpora allata cease. The corpora allata then become active and induce egg maturation. In both cases the brain seems to be the regulating center for the function of the corpora allata, and there is evidence that also in other insect species the brain functions as a controlling center for a variety of different endocrine functions (Wigglesworth, 1934; Scharrer, 1958).

Extrinsic factors such as mating and parturition, are particularly important for the proper control of the reproductive cycles in *Diploptera*. In other species of cockroaches, at least one of these factors, namely mating, is involved in reproduction. In *Periplaneta americana*, for instance, mating accelerates growth of the eggs and consequently more eggs are produced during the animal's lifetime if it is permitted to mate (Griffiths and Tauber, 1942; Roth and Willis, 1956). In this species, however, the influence of mating seems less pronounced than in *Diploptera*. The same is true for *Leucophaea maderae* where mating also enhances the activity of the corpora allata during the first pre-oviposition period. Egg maturation in this species is accelerated by mating only by about one-third of the period required in virgins (Engelmann, unpublished data) in contrast to the observation in *Diploptera* where the acceleration of egg maturation upon mating amounts to at least five times; on the average even more.

In the second and subsequent reproductive cycles of *Diploptera* the stimuli normally occurring in parturition are essential in replacing those of mating. In *Periplaneta* and in *Leucophaea*, on the other hand, no comparable stimulation seems to be required as is shown by the following observations. In females of *Periplaneta*, isolated after their first mating, egg maturation in successive batches took only slightly longer than that in females which were continuously kept with males (Griffiths and Tauber, 1942). In this species parturition does not occur and, therefore, mating would have to replace the effect of giving birth as described in *Diploptera*. In contrast to the situation in *Diploptera*, mating in *Leucophaea* after removal of the egg case did not shorten the time needed for egg maturation (Engelmann, unpublished data). At the present time no explanation can be given for the

fact that in *Periplaneta* and in *Leucophaea* mating influences egg maturation only during the first pre-oviposition period and not thereafter. From this it seems that *Diploptera* is unique among cockroaches with respect to the degree to which stimulation of the corpora allata and consequently activation of the ovaries depends on afferent nervous stimuli.

In females of *Diploptera* the normal mating act can be simulated by "artificial mating," a fact which indicates that mechanical stimulation of parts of the genital apparatus is the essential factor involved. It might be worth mentioning in this connection that in some mammals such as the ferret, rabbit, weasel, cat, mating likewise is essential for the completion of egg maturation and ovulation. In the cat, ovulation can be brought about even by artificial stimulation of the cervix uteri by means of a glass rod (Greulich, 1934; Porter *et al.*, 1957). Here, upon mechanical stimulation of an area in the genital apparatus, the brain activates the hypophysis, which in turn induces ovulation by releasing gonadotropic hormones; this is analogous to the situation in *Diploptera*. One should point out that in the cat maturation of the egg nucleus and the rupture of the follicle is induced by mating, whereas in *Diploptera* it results in deposition of yolk in the oocytes. These are two fundamentally different processes related to different reproductive mechanisms, but both are induced by mating and both finally lead to ovulation.

In *Diploptera*, as in *Leucophaea*, we observe alternating activity and inactivity of the corpora allata correlated with periods of egg maturation and of quiescence of the ovaries. In this respect the reproductive patterns of two related species are strikingly similar, but as was shown the regulation of these reproductive cycles is achieved in different ways. In the female of *Diploptera* the corpora allata seem to be maintained at a low level of activity. For every activation of these glands, sufficiently high to induce egg maturation within a normal period of time, a stimulation of the genital apparatus has to take place. On the other hand, in most virgins of *Leucophaea* the corpora allata appear to be only slightly inhibited during the first pre-oviposition period. During pregnancy, in *Diploptera*, a substance furnished by the mature eggs in the brood sac inhibiting the corpora allata seems not to be the primary restraining principle. In pregnant *Leucophaea*, however, such a substance originating in the eggs plays a dominant role in the control of the corpora allata (Engelmann, 1957). Thus, in both related species we find humoral as well as nervous afferent stimuli involved in the regulation of reproduction. They seem to operate independently of each other. They are effective in both species, but in *Diploptera* the nervous factor is the more important, whereas in *Leucophaea* the reverse is the case.

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SUMMARY

1. In the viviparous roach, *Diploptera*, the factors controlling the maturation of the eggs were studied during an interval encompassing the first pre-oviposition, first pregnancy, and second pre-oviposition periods. During egg maturation the corpora allata become active as judged by histological criteria (increase of cyto-

plasmic content); they become and stay inactive during pregnancy. Ovarian inactivity after allatectomy also demonstrates that active corpora allata are responsible for the deposition of yolk in the oocytes. In addition, the corpora allata control the activity of the accessory sex glands, and of an as yet unknown type of cell presumably located in the bursa copulatrix, the secretion of which facilitates the extrusion of the spermatophore.

2. In virgins, the brain inhibits the function of the corpora allata by way of the nervi corporis allati, and thus prevents or delays the maturation of the eggs. Severance of these nerves results in a sustained activity of the corpora allata with the result that several successive batches of eggs mature.

3. In females which have not yet borne young, mating is essential for a normal rate of egg maturation. The effect of mating can be replaced by parturition which provides adequate stimuli to induce egg maturation. Thus, in an animal that has just given birth, mating does not step up the growth rate of the eggs. The stimuli exerted by mating or parturition are transmitted to the corpora allata by way of the ventral nerve cord and the brain.

4. Artificial mating (mechanical stimulation with a small glass spermatophore) also results in activation of the corpora allata and in subsequent maturation of the eggs. Similarly, excision of the gonapophyses induces egg maturation, presumably because the afferent nerves are stimulated by this procedure. It seems likely that sensory receptors on the gonapophyses and possibly on other parts of the genital apparatus receive the stimuli exerted by mating and parturition.

5. The corpora allata of adult females of *Diploptera* are restrained to the extent that egg maturation occurs only after considerable delay unless this inhibition is eliminated by appropriate afferent stimuli. Complete inhibition of the corpora allata, and thus of the ovaries, during pregnancy is achieved by the eggs developing in the brood sac.

6. Ovariectomy does not result in sustained activity of the corpora allata as is the case in some other insect species.

LITERATURE CITED

- ENGELMANN, F., 1957. Die Steuerung der Ovarfunktion bei der ovoviviparen Schabe *Leucophaea maderae* (Fabr.). *J. Ins. Physiol.*, **1**: 257-278.
- ENGELMANN, F., AND M. LÜSCHER, 1956. Die hemmende Wirkung des Gehirns auf die Corpora allata bei *Leucophaea maderae* (Orthoptera). *Verh. dtsh. zool. Ges. Hamburg*, **1956**: 215-220.
- GREULICH, W. W., 1934. Artificially induced ovulation in the cat (*Felis domestica*). *Anat. Rec.*, **58**: 217-224.
- GRIFFITHS, J. T., AND O. E. TAUBER, 1942. Fecundity, longevity and parthenogenesis of the American roach, *Periplaneta americana* L. *Physiol. Zool.*, **15**: 196-209.
- VON HARNACK, M., 1958. Histophysiological studies on the corpus allatum of *Leucophaea maderae*. II. The effect of starvation. *Biol. Bull.*, **115**: 521-529.
- VON HARNACK, M., AND B. SCHARER, 1956. A study of the corpora allata of gonadectomized *Leucophaea maderae* (Blattaria). *Anat. Rec.*, **125**: 558.
- JOHANSSON, A. S., 1955. The relationship between corpora allata and reproductive organs in starved female *Leucophaea maderae* (Blattaria). *Biol. Bull.*, **108**: 40-44.
- JOHANSSON, A. S., 1958. Relation of nutrition to endocrine-reproductive functions in the milkweed bug *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). *Nytt Mag. Zool.*, **7**: 1-132.
- LÜSCHER, M., AND F. ENGELMANN, 1955. Ueber die Steuerung der Corpora allata-Funktion bei der Schabe *Leucophaea maderae*. *Rev. Suisse Zool.*, **62**: 649-657.

- PORTER, R. W., E. B. CAVANAUGH, B. V. CRITCHLOW AND CH. H. SAWYER, 1957. Localized changes in electrical activity of the hypothalamus in estrous cats following vaginal stimulation. *Amer. J. Physiol.*, **189**: 145-151.
- ROTH, L. M., AND E. R. WILLIS, 1955. Intra-uterine nutrition of the "beetle-roach" *Diploptera dytiscoides* (Serv.) during embryogenesis, with notes on its biology in the laboratory (Blattaria: Diplopteridae). *Psyche*, **62**: 55-68.
- ROTH, L. M., AND E. R. WILLIS, 1956. Parthenogenesis in cockroaches. *Ann. Ent. Soc. Amer.*, **49**: 195-204.
- SCHARRER, B., 1946. The relationship between corpora allata and reproductive organs in adult *Leucophaea maderae* (Orthoptera). *Endocrinol.*, **38**: 46-55.
- SCHARRER, B., 1952. Neurosecretion. XI. The effects of nerve section on the intercerebralis-cardiacum-allatum system of the insect *Leucophaea maderae*. *Biol. Bull.*, **102**: 261-272.
- SCHARRER, B., 1958. Neuro-endocrine mechanisms in insects. 2. Internat. Sympos. Neurosekretion (Lund, 1957). Springer-Verlag, Berlin-Göttingen-Heidelberg, 79-84.
- WHARTON, M. L., AND D. R. A. WHARTON, 1957. The production of sex attractant substance and of oothecae by the normal and irradiated American cockroach, *Periplaneta americana* L. *J. Ins. Physiol.*, **1**: 229-239.
- WIGGLESWORTH, V. B., 1934. The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and 'metamorphosis.' *Quart. J. Micr. Sci.*, **77**: 191-222.
- WILLIS, E. R., G. R. RISER AND L. M. ROTH, 1958. Observations on the reproduction and development in cockroaches. *Ann. Ent. Soc. Amer.*, **51**: 53-69.